UNITED NATIONS

SECRETARIAT

ST

Distr.
GENERAL

ST/SOA/SER.J/21
30 October 1975

Original: ENGLISH

SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM [1]

No. 21

Rapid semi-quantitative spot test for determination of thebaine and differentiation of Papaver bracteatum from P. orientale and P. pseudo-orientale [2]

by: P.G. Vincent, C.Z. Bare and W.A. Gentner,
United States Department of Agriculture,
Agricultural Research Service,
Beltsville, Maryland 20705, U.S.A.

1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Third Working Group on Papaver bracteatum (document ST/SOA/SER.J/15), it was recommended that a rough screening method should be developed for the determination of thebaine.

GE. 75-12087

The section Oxytona of the genus Papaver consists of three distinct species which occur in Turkey (1), i.e. P. bracteatum (2n=14), P. orientale (2n=28) and P. pseudo-orientale (2n=42). Differentiation between the species has been limited to observing the plant at time of flowering, analyzing plant tissue for alkaloid content, determining the number of chromosomes, and often combinations of these. In each instance problems limit its applicability to species differentiation, e.g. flowering occurs within a limited time frame, alkaloid determination requires somewhat sophisticated equipment, and chromosome determinations require especial expertise and are laborious.

At the Third Working Group on P. bracteatum it was recommended that a rapid field test for thebaine be forthcoming. One such test has already been submitted for publication in the United Nations ST/SOA/SER.J/.. (Küppers, F.E.J.M. and P.G. Vincent. Field test for thebaine analysis, 1975). The test involves the use of thin-layer chromatography using reference thebaine standards and two solvent systems.

The method described herein exploits the fact that the principal alkaloid found in P. bracteatum is thebaine which gives a rather specific colour reaction with molybdate in concentrated sulfuric acid.

Colour was determined using the Inter-Society Colour Council-National Bureau of Standards (ISCC-NBS) Colour-Name Charts Illustrated with Centroid Colours (3, 4). Colours are described using ISCC-NBS designation followed by Munsell renotation (4) in parenthesis.

In 1866, Fröhde introduced his reagent as a test for morphine (5). The reagent is made by dissolving 0.5 g of ammonium or sodium molybdate in 100 ml concentrated sulfuric acid. This reagent should not be confused with "Buckingham's reagent" which contains 10-15 g molybdate per 100 ml. Fröhde's reagent is sensitive to approximately 1 µg of alkaloid. The alkaloids found in the genus Papaver give some specific colour reactions in the presence of a few drops of Fröhde's reagent. For example, morphine plus reagent initially produced a very deep purple red, ISCC-NBS No. 257 (6.8 RP 1.7/8.0) followed by dark olive, ISCC-NBS No. 108 (8.9 Y 2.4/3.1) which faded to medium red brown, ISCC-NBS No. 43 (9. OR 3.4/5.2) and finally to strong green, ISCC-NBS No. 141 (5.8 G 4.4/8.7). Codeine plus reagent gave an initial deep yellow green colour, ISCC-NBS No. 132 (0.9 G 3.5/9.0) followed by a dark olive green, ISCC-NBS No. 126 (8 OGY 2.2/3.6). Papaverine in

the presence of reagent gave a brilliant yellow green, ISCC-NBS No. 116 (4.0 GY 8.2/9.1). Fröhde's reagent plus isothebaine initially produced a dark blue, ISCC-NBS No. 183 (2.2 PB 1.7/5.5) which immediately went to dark olive brown, ISCC-NBS No. 96 (2.6 Y 1.9/2.2). Initial colour of oripavine plus reagent was light olive, ISCC-NBS No. 106 (8.2 Y 5.1/5.6) which turned to medium olive, ISCC-NBS No. 107 (7.6 Y 3.8/5.4). Narcotine plus reagent was grey green at first, ISCC-NBS No. 150 (8.8 G 4.5/1.8) then became dark green, ISCC-NBS No. 146 (6.6 G 2.8/4.6). Orientalidine plus reagent was medium grey ISCC-NBS No. 265 (3.3 GY 5.4/0.1) and alpinigenine was grey violet, ISCC-NBS No. 215 (1.2 P 3.3/3.9). Narceine plus reagent was blue grey, ISCC-NBS No. 191 (8.9 B 5.5/0.9). Thebaine plus reagent gave a deep orange, ISCC-NBS No. 51 (4.1 YR 5.1/11.3). The colour reactions are not specific for alkaloids. Other substances such as aromatic amines, phenols or ethers of phenols yield different colours. However, the colours and sequence of colour change seem to be, in general, specific for each substance.

Method

White enamel spot plates, 9 x 12 cm, having 12 wells were employed throughout. Fröhde's reagent was prepared by dissolving 0.5 g ammonium molybdate in 100 ml concentrated sulfuric acid. Aliquots containing 1 to 120 µg of thebaine were pipetted into the wells. Approximately 10 drops of Fröhde's reagent were added to each well. The colour from the reaction of the known concentration of thebaine was compared to the colour of the unknown and a visual semi-quantitative estimation of thebaine from P. bracteatum could thus be made. Care must be taken to avoid introduction of moisture into the reagent or during the testing itself, particularly under high humidity conditions. Moisture will obviate the colour reactions and, in most cases, is responsible for the sequence of colour changes observed.

This procedure could be used prior to analyses by more sophisticated methods such as gas-liquid chromatography or high performance liquid chromatography when screening extracts for their thebaine content.

Fröhde's reagent can also be added directly to latex from species of Papaver. Latex may be derived from excised leaves, roots or capsules. For qualitative analysis, latex from P. somniferum, P. bracteatum, P. orientale, P. pseudo-orientale and P. alpinium excised leaf tissue were spotted in the wells of the plate and allowed to dry. Ten drops of Fröhde's reagent were added to each well, the plate gently rotated, and the colour development noted. The initial colour reactions were, for P. somniferum latex a deep purple red, ISCC-NBS No. 259 (7.1 RP 2.7/6.0), followed by a medium red brown (described above), and finally a dark olive green (described above). P. orientale latex with reagent gave an initial colour of very dark yellow green, ISCC-NBS No. 138 (0.3 G 1.8/4.3), followed by brown black, ISCC-NBS No. 65 (7.8 YR 0.6/0.9). P. pseudo-orientale with reagent gave an initial colour of very dark red, ISCC-NBS No. 17 (4.2 R 1.2/4.8), followed by a dark red brown, ISCC-NBS No. 44 (9.6 R 1.3/3.6). P. alpinium latex with reagent yielded similar colours as those described for P. pseudo-orientale. P. bracteatum latex with reagent was initially very deep red, ISCC-NBS No. 14 (6.5 R 1.7/8.4), followed by slight red brilliant, ISCC-NBS No. 40 (0.3 YR 3.1/9.9).

Spot plates were prepared by Dr. J. Duke, Plant Taxonomy Laboratory, USDA, ARS, Beltsville, Maryland 20705, as a "blind" test for discriminating between the Papaver species as well as other plant tissue. Plants evaluated were P. bracteatum, P. orientale, P. pseudo-orientale, P. pseudo-orientale c.v. "May Curtis", Eschscholzia californica (California poppy), P. lateritium, Asclepias syriaca (milk weed), Lactuca sativa (lettuce) and Euphorbia sp. (mole plant). Results of the reaction of these plant latex (or sap) materials with Fröhde's reagent are shown in Table 1. In all cases we were able to distinguish the P. bracteatum latex from other expressed fluids with the possible exception of P. fugax. Species differentiation is probably due to the fact that 95-98% of the alkaloids in the P. bracteatum latex consist of thebaine, whereas the other Papaver species contain different alkaloids or thebaine in conjunction with other alkaloids. The effect of the presence of other alkaloids is to quench the slight red brilliant colour typically produced in the bracteatum samples. In addition to the alkaloids, other phenolic or aromatic amine substances are undoubtedly reacting with the reagent and producing specific colours.

A semi-quantitative estimation of thebaine content of the latex may be made in the following manner. A capillary pipette (1-5 µl) is used to collect the latex. The latex may be directly transferred to the spotting well. Some loss will occur due to adhesion of the material to the pipette. During quantitative determinations the pipette plus latex can be dropped into a screw cap test tube containing a given volume of methanol. The test tube containing the methanol together with the pipette and the latex is then agitated for several minutes. An aliquot of the resulting solution, usually 100 µl from a total volume of 1 ml, is placed in the spotting well and allowed to dry. The advantages of the methanol method are rapid dehydration of latex, removal of most substances which might interfere with the colour reaction, and a more quantitative extraction especially if the methanol is made slightly acidic prior to extraction with a volatile organic acid. It should be noted that the volume of latex in the plant will change throughout the day as a function of moisture loss or gain. Therefore, caution must be used when extrapolating data derived from pipetted samples. Leaf tissue subjected to direct application of Fröhde's reagent gave variable colour reaction and is not recommended for thebaine determination.

Thus, a comparatively simple, portable and inexpensive test for thebaine determination is presented. The test also permits the user to distinguish P. bracteatum from P. orientale and P. pseudo-orientale by exploiting the fact that the principal alkaloid in P. bracteatum is thebaine.

References

1. Goldblatt, Peter. 1974. Biosystematic studies in Papaver section Oxytona. Annals of the Missouri Botanical Garden, 61 (2):254-296.

2. Kelly, Kenneth L. 1965. A universal colour language. Colour Engineering, 3 (2):2-7.

3. ISCC-NBS Centroid Colour Charts, Standard sample No. 2106 obtainable from the Office of Standard Reference Materials, Room 215, Chemistry Building, National Bureau of Standards, Washington, D.C. 20234. $3.00 per set.

4. Munsell, A.H. A colour notation, 11th edition, Munsell Colour Company Inc., Baltimore, Maryland, 1961.

5. Fulton, C.C. 1938. Fröhde's reagent: A reagent for morphine and for other phenolic compounds. J. Lab. clin. Med., 23:625-630.

Table 1

Colour reactions of different plant latex or sap with Fröhde's reagent

Plant	Final colour 1/
P. bracteatum latex	slight red brilliant
P. pseudo-orientale latex	dark red brown
P. pseudo-orientale c.v. "May Curtis"	dark red brown
P. fugax latex	dark olive green
P. lateritium latex	dark grey olive green
Eschscholzia californica (California poppy)	dark grey olive green
Asclepias syriaca (milk weed)	grey olive green
Lactuca sativa (lettuce)	brown orange
Euphorbia sp. (mole plant)	brown orange

1/ After exposure to reagent for 5 minutes; refer to ISCC-NBS numbers given in the text for colour designation.